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Circular RNAs Biogenesis, Regulatory Mechanisms, and Their Future Applications in Laboratory Medicine

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A B S T R A C T

Introduction: Circular RNAs (circRNAs)- once considered splicing artifacts- are covalently closed RNA molecules generated through back-splicing, lacking 5′ caps and 3′ poly(A) tails, which confers them with remarkable stability. Recent high- throughput sequencing and bioinformatic advances have uncovered diverse biogenesis pathways and functions of circRNAs, including acting as microRNA sponges, interacting with RNA-binding proteins, forming RNA-DNA hybrids, undergoing N6-methyladenosine modification, and even being translated into peptides.

Aim: This review summarizes recent insights into circRNA biogenesis, mechanisms of action, and emerging biomedical applications.

Discussion: Their dysregulation has been implicated in various diseases- cancer, neurodegeneration, cardiovascular disorders- and they show promise as diagnostic biomarkers and therapeutic targets. Moreover, engineered circular RNAs are being explored for vaccine design and RNA-based therapies.

Conclusion: This review summarizes recent insights into circRNA biogenesis, mechanisms of action, and emerging biomedical applications.

Keywords: circular RNA, circRNA, back-splicing, microRNA sponge, RNA- binding proteins, m^6A, peptide translation, biomarker, RNA therapy

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Introduction

For decades, RNA was primarily seen as a mere messenger (mRNA) or structural molecule (rRNA/tRNA). However, the shift from the "junk RNA misconception" to “functional RNAs” has occurred because the discovery of functional non-coding RNAs (ncRNAs)—like microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs)—revealed that RNA plays dynamic roles in gene expression, cell signalling, and disease (1). Although early studies dismissed circRNAs as accidental splicing byproducts with no biological role (once considered splicing artifacts, as circRNAs were initially discovered in the 1970s during electron microscopy studies of RNA viruses but were considered mainly splicing errors for decades), advances culminating in next-generation sequencing and bioinformatics were incorporated into high-throughput RNA sequencing (RNA-seq) tools in the 2010s, which enabled genome-wide detection of back-splice junctions unique to circRNAs and uncovered their ubiquity, conservation, and functions (e.g., miRNA sponging, protein interaction) (2, 3). This technological progress revealed that circRNAs are abundant, conserved, and dynamically regulated in various physiological and pathological conditions (1, 3). The non-coding RNA revolution has transformed our understanding of RNA biology, revealing a universe of regulatory molecules beyond protein-coding genes. circRNAs are now recognized as key players in gene regulation, with profound implications for health and disease. These findings highlight how scientific progress redefined circRNAs and made them a hot topic (4, 5). In the current review, mechanisms (biogenesis, regulation) and some biomedical impacts (mechanisms of action, medical applications) of circRNAs are discussed.

circRNAs structural properties

Unlike linear RNAs, which have free 5’ and 3’ ends, circRNAs form a single-stranded continuous loop where the 3’ end is covalently linked to the 5’ end (via a phosphodiester bond). This phenomenon bestows them degradation resistance against exonucleases (e.g., RNase R), making them highly stable. CircRNAs lack a poly(A) tail or 5’ cap, distinguishing them from mRNAs. These are formed through reverse splicing, in which the non-canonical splicing event occurs by the ligation of a downstream 5' splice site (donor) to an upstream 3' splice site (acceptor), i.e., the reverse of linear RNA splicing by the spliceosome (6, 7).

Circular RNAs Bio-distribution

While circRNAs are the molecules that are most extensively studied in eukaryotes, particularly in animals and plants, there is a growing body of evidence of their presence in other domains of life. In prokaryotes, circRNA-like structures have been observed, even if their biogenesis and functions remain less clear. circRNAs have been detected and associated with immune modulation and viral replication in viruses, especially genomic DNA viruses such as Epstein–Barr virus (EBV) and hepatitis B virus (HBV). The existence of virus-derived circRNAs suggests roles in host-pathogen interactions. In eukaryotes, circRNAs are highly conserved and demonstrate tissue-specific expression, highlighting their significance in cellular processes such as gene expression regulation (8, 9). Recent studies identified mitochondrial circRNAs (mecciRNAs), which are derived from mitochondrial transcripts, and these mecciRNAs may play roles in mitochondrial function and communication with the nucleus. Moreover, some circRNAs can encode proteins or peptides, which are classified as coding circRNAs, especially those with internal ribosome entry sites (IRES) or N6-methyladenosine (m^6A) modifications that facilitate cap-independent translation. The diverse type of circRNAs reflects their multifaceted roles in post-transcriptional regulation and emphasizes the growing complexity of the non-coding RNA landscape (1, 8).

Biogenesis of circRNAs

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Biogenesis of circRNAs occurs in two main mechanisms: i) Canonical back-splicing and

ii) non-canonical or alternative back-splicing. Moreover, these mechanisms are tightly regulated. Table 1 shows a comparison of these two pathways of circRNA biogenesis(10).

Canonical Back-splicing

Canonical back-splicing is the fundamental mechanism responsible for generating circRNAs (Figure 1). Unlike canonical splicing, which joins exons in a linear sequence, during back-splicing, a downstream 5' splice donor covalently joins an upstream 3' splice acceptor in a reverse order compared to linear splicing. This forms a closed loop, i.e., circRNA, but the sequence itself remains in the same 5'→3' direction as in the parent RNA. This reverse splicing event results in the formation of a circular RNA molecule that lacks 5′ caps and 3′ poly(A) tails (1, 11). The phosphodiester bonds circularly connect the ends in a circular fashion, but the individual nucleotides retain their original 5'→3' orientation within the circRNA. The sequence remains identical to the genomic sense strand, just circularized. Since the nucleotide direction is preserved, circRNAs can still bind miRNAs or proteins in the same sequence context as the linear RNA. Unlike reverse transcription or inversion, the sequence is not reversed or complemented (12). Key features of canonical back-splicing include i) strict intronic requirements that depend on reverse complementary sequences, e.g., Alu repeats, in flanking introns that bring splice sites close together; ii) it may involve RNA-binding proteins (RBPs), e.g., QKI, FUS, and MBL, that stabilize the loop RBPs; iii) spliceosome-dependency, which requires the canonical spliceosome machinery (snRNPs: U1, U2, U4/U6, and U5); and iv) predictable circRNA output (typically produces single-exon circRNAs (ecircRNAs) or exon-intron circRNAs (EIciRNAs). An example of canonical back-splicing is circCDR1as (ciRS-7), which is formed via Alu-repeat-mediated back-splicing in the CDR1 locus (12-14)

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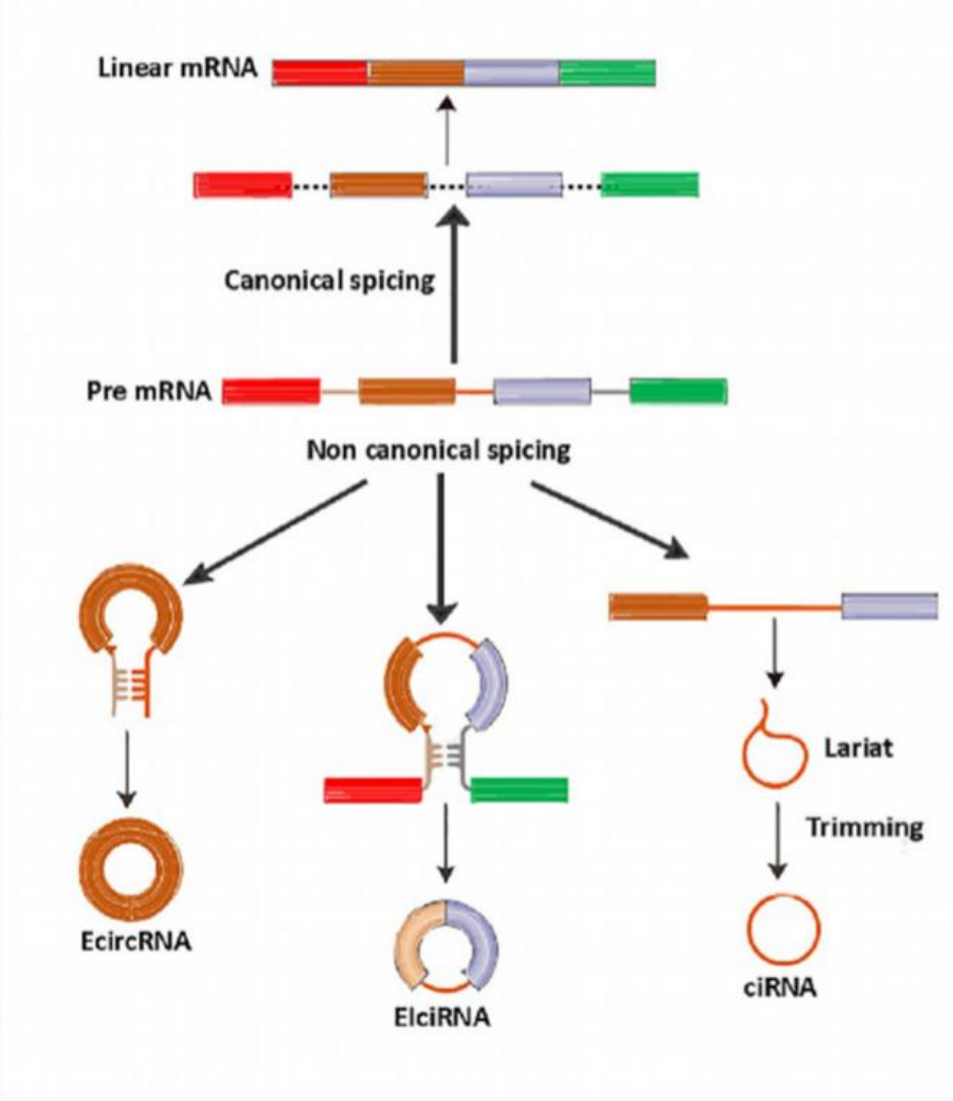


Figure 1. mRNA processing. The cell can synthesize different mRNAs using canonical and alternative (non-canonical) back-splicing mechanisms.

Alternative (Non-canonical) Back-Splicing

Non-canonical back-splicing events differ from the canonical mechanism, often producing atypical circRNAs (Figure 1). Alternative back-splicing refers to the processes where a single gene can produce multiple circRNA isoforms due to variations in back-splice junction selection. This is analogous to the alternative splicing in linear RNAs; however, it occurs in a circularization manner (6). CircRNAs are generated via non-canonical splicing, where a downstream 5′ splice donor site ligates to an upstream 3′ splice acceptor. Thus, a single pre-mRNA can generate different circRNAs by using alternative splice sites (different 5' or 3' splice donors/acceptors) (3). Including or excluding exons, introns, or retained introns (forming exonic, intronic, or EIciRNAs). Alternative back-splicing can synthesize exonic (ecircRNA), intronic (ciRNA), or exon-intronic (EIciRNA) sequences (8). The produced circRNAs are broadly categorized based on their structural composition and genomic origin, including: i) Exonic circRNAs (ecircRNAs), which are composed solely of exonic sequences and are predominantly found in the cytoplasm, where they can function as microRNA sponges or interact with RNA-binding proteins; ii) ciRNAs derived from lariat introns that escape debranching and are often localized in the nucleus to regulate transcription; and iii) EIciRNAs, which retain both

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exonic and intronic sequences and participate in gene expression regulation within the nucleus (1, 3, 8). In addition, iv) Fusion circRNAs (f-circRNAs) and v) Read-through circRNAs (rt-circRNAs) have been identified, particularly in cancer, often arising from chromosomal translocations or complex splicing events (3). Mechanisms leading to alternative back-splicing include i) variable flanking intronic sequences, which are complementary sequences (e.g., Alu repeats) that may promote different back-splice pairings; ii) RBPs, such as proteins like Quaking (QKI), FUS, or MBL, that can determine which exons to be circularized; and iii) competition with linear splicing, as some splice sites may be used for either linear or circular RNA production (15, 16). Alternative back-splicing has a variety of biological implications. Producing different circRNA isoforms may have distinct miRNA/protein-binding capacities, and this catachresis bestows circRNAs functional diversity. Some circRNA isoforms are enriched in specific tissues (e.g., brain muscle) and indeed possess a tissue-specific expression pattern. Therefore, aberrant alternative back-splicing is linked to cancer, neurodegeneration, and other disorders. Interestingly, alternative back-splicing increases the complexity of the circRNA landscape, allowing a single gene to produce functionally diverse circular RNAs. This process is regulated by splicing machinery, RBPs, and genomic architecture, contributing to cell-type-specific gene regulation (16). There are five main mechanisms in alternative splicing to produce a variety of circRNAs: The first mechanism is lariat-driven circularization, which occurs when a linear exon-containing lariat intron escapes debranching and undergoes internal back-splicing, generating circular intronic RNAs (ciRNAs). The second mechanism is RBP-mediated circularization without complementary sequences, which is mediated by RBPs (e.g., FUS/Fused in Sarcoma, EWSR1/Ewing Sarcoma RNA-binding protein 1) that dimerize to bridge distant splice sites, generating circRNAs from intron-poor genes such as circMAN1A2 in cancer (17). The third mechanism is exon skipping or alternative splice site usage that is mediated by alternative 5’/3’ splice sites to create variable-length circRNAs, generating multiple circRNA isoforms from the same gene (e.g., circHIPK3 variants). The fourth mechanism is trans-splicing of separate transcripts in which back-splicing occurs between exons from different pre-mRNAs and the output is fusion circRNAs (f-circRNAs), often in cancers with chromosomal translocations. An example is f-circM9, which is derived from the MLL-AF9 fusion gene in leukemia (6, 17).

Table 1. Comparison of two pathways for circRNA biogenesis.

Feature	Canonical back-splicing	Alternative back-splicing
Splice Sites	Strict 5’ donor + 3’ acceptor	Variable or non-canonical sites
Intronic Requirement	Complementary sequences needed	May lack complementary motifs
Spliceosome	Required	Sometimes bypassed (e.g., lariats)
Regulation	Tissue-specific RBPs	Stress-induced or pathological
Output	Stable ecircRNAs/EIciRNAs	ciRNAs, f-circRNAs, variable isoforms

Biogenesis Regulation

CircRNA biogenesis is regulated by tissue-specific splicing factors and epigenetic modifications (e.g., histone marks, DNA methylation). Circular RNA (circRNA) biogenesis is tightly regulated by tissue-specific splicing factors, which determine where, when, and how circRNAs are produced. These factors influence back-splicing efficiency, circRNA

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stability, and functional diversity across different cell types. There are some key splicing factors in circRNA formation, such as muscle-binding proteins (e.g., MBL/MBNL), Quaking (QKI), FUS and EWSR1, and hnRNPs (e.g., hnRNP L, hnRNP A1). Table 2 shows some tissue-specific regulation examples for circRNA biogenesis (18, 19).

Recent high-throughput sequencing studies have demonstrated that back-splicing is a tightly regulated, non-random process that occurs in a cell type- and tissue-specific manner (1, 20). One tissue-specific splicing factor is MBL/MBNL proteins, which bind to intronic flanking sequences of circRNA-forming exons, promoting loop formation. For example, MBL (Muscleblind) regulates circMbl in Drosophila and mammalian muscle/heart tissue (21). QKI is another tissue-specific splicing factor that works by recognizing QKI response elements (QREs) in introns and bridging distant splicing sites.

They are highly active in endothelial and epithelial cells. For example, QKI upregulation in epithelial-mesenchymal transition (EMT) increases circZKSCAN1 production (22).

FUS and EWSR1 are tissue-specific splicing factors that act by dimerization to tether introns, enabling back-splicing in neurons and cancer cells. For example, FUS-dependent circRNAs (e.g., circTAF15) are abundant in neurons from patients with amyotrophic lateral sclerosis (23). Another group of splicing factors are hnRNPs (e.g., hnRNP L, hnRNP A1), which exert their effects as repressors or enhancers of back-splicing depending on cellular context. For example, hnRNP L suppresses circPABPN1 in HeLa cells but promotes it in hepatocytes. These mechanisms exerted by tissue-specific splicing factors to regulate circRNA biogenesis may have pathogenic consequences, as loss or gain of function of these splicing factors can affect the fate of cells and tissues. Recent studies have shown that QKI deletion in glioblastoma reduces tumor-suppressive circRNAs, and FUS mutations in ALS disrupt neuronal circRNA profiles. Meanwhile, tissue-specific splicing factors may have therapeutic potential, as targeting splicing factors (e.g., antisense oligonucleotides to modulate QKI) could restore circRNA balance. In this regard, one of the treatment options is tissue-specific circRNAs to be engineered for organ-targeted therapies (24, 25).

Table 2. Tissue-specific regulation examples for circRNA biogenesis.

Tissue/Cell type	Splicing factor	circRNA target	Functional outcome
Neurons	FUS, NOVA1	circHomer1	Synaptic plasticity
Heart	RBM20	circSLC8A1	Cardiomyopathy regulation
Liver	hnRNP A1	circHIPK3	Glucose metabolism
Cancer (Epithelial-mesenchymal transition)	QKI	circZKSCAN1	Metastasis suppression

As mentioned, circRNA biogenesis is not only controlled by splicing factors but also fine-tuned by epigenetic modifications, including histone post-translational modifications (PTMs) and DNA methylation. These modifications influence chromatin accessibility, transcriptional activity, and splice site selection, ultimately impacting circRNA production. Histone marks near circRNA-forming loci can either promote or suppress back-splicing by modulating RNA polymerase II (Pol II) elongation and spliceosome recruitment (26). An activating mark that drives circRNA biogenesis is H3K36me3 (trimethylation of histone H3 at lysine 36), which is enriched in the exons of actively transcribed genes and recruits splicing factors (e.g., SR proteins) to facilitate back-splicing. High H3K36me3 levels correlate with circHIPK3 production in cancer cells (27). Another activating mark is H3K4me3

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(trimethylation of histone H3 at lysine 4), which marks transcription start sites (TSS) of circRNA host genes and enhances Pol II processivity, increasing back-splicing opportunity. CircANRIL (linked to atherosclerosis) is upregulated in H3K4me3-enriched regions (28, 29). Meanwhile, there are repressive marks that inhibit circRNA biogenesis. One of these repressive marks is H3K27me3 (trimethylation of histone H3 at lysine 27), and its action is mediated by polycomb repressive complex 2 (PRC2), which condenses chromatin, blocking RNA polymerase II elongation and splicing factor access. In this regard, loss of H3K27me3 in glioblastoma increases oncogenic circSMARCA5 (28).

Another repressive mark (inhibition of circRNA biogenesis) is of H3K9me2/3 (methylation of histone H3 at lysine 9), which is associated with heterochromatin silencing and prevents

back-splicing by restricting transcriptional activity. In this regard, circDYM (neuroprotective) is suppressed in Alzheimer's due to elevated H3K9me3 (28).

Another way of circRNA biogenesis regulation is regulation of DNA methylation (5-methyl-cytosine or 5mC) at Cytosine-phosphate-Guanine (CpG) sites. DNA methylation at CpG sites near splice positions can block or enhance circRNA formation. Promoter methylation, which suppresses circRNA production by hypermethylation, silences host gene transcription, reducing circRNA precursors. For example, circFNDC3B expression is downregulated in colorectal cancer due to promoter methylation. On the other hand, intronic methylation, which modulates back-splicing efficiency by hypomethylation in flanking introns, exposes complementary sequences (e.g., Alu repeats), facilitating back-splicing. For example, circCCDC66 is upregulated in lung cancer due to intronic hypomethylation (30, 31). Some of the epigenetic effects on the circRNA biogenesis have been shown in Table 3.

Table 3. Epigenetic- circRNA crosstalk in disease.

Disease	Epigenetic Change	circRNA Affected	Outcome
Breast Cancer	H3K27ac gain	circTADA2A	Enhances tumor growth
Neurodegeneration	H3K9me3 accumulation	circDYM ↓	Linked to synaptic dysfunction
Atherosclerosis	H3K4me3 at ANRIL locus	circANRIL ↑	Protects against plaque formation

In addition, post-transcriptional modifications also play roles in circRNA synthesis. One of these modifications is the m^6A moiety of RNA that has been shown to facilitate the back-splicing process by recruiting specific reader proteins (10, 11).

Epigenetic regulation of circRNA biogenesis may have diagnostic and therapeutic implications, as epigenetic drugs known as histone deacetylase (HDAC) inhibitors (e.g., Vorinostat) can increase H3K9ac, boosting neuroprotective circRNAs, and DNA methyltransferase (DNMT) inhibitors (e.g., 5-Azacytidine) may restore tumor-suppressive circRNAs. Albeit, lack of specificity (global epigenetic changes may have off-target effects) is a primary challenge in this regard. Another question that remains unanswered is whether epigenetic signatures of circRNAs can serve as biomarkers for disease progression.

Mechanisms of Action

T CircRNAs have diverse and important mechanisms of action (Figure 2) in regulating gene expression and cellular processes.

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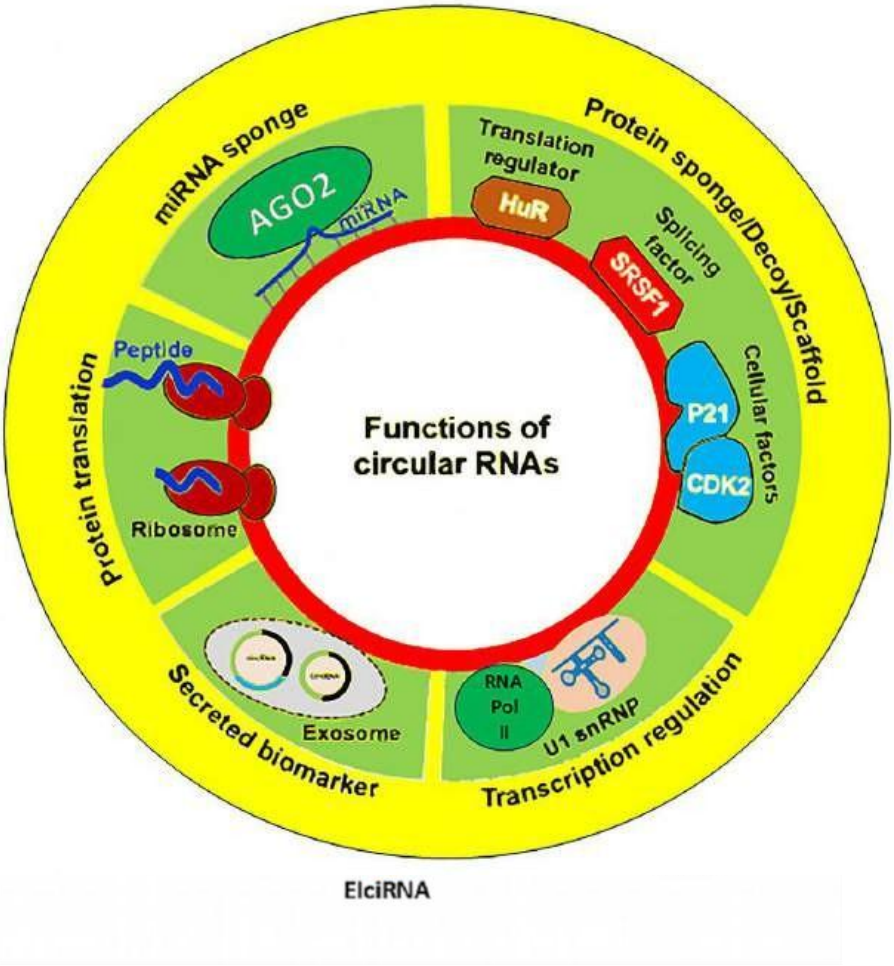


Figure 2. mRNA processing. The cell can synthesize different mRNAs using canonical and alternative (non-canonical) back-splicing mechanisms.

Mechanisms of Action of circRNAs can be categorized as follows:

miRNA Sponging (Competitive Endogenous RNA Activity)

A key mechanism by which circRNAs exert their functions is acting as miRNA sponges. A single circRNA can efficiently sequester specific miRNAs by using multiple miRNA binding sites, thereby preventing them from repressing target mRNAs with miRNA binding. The typical example is ciRS-7/CDR1as, which contains over 70 binding sites for miR-7, and this sponge activity has been implicated in regulating pathways critical for cancer and neurodegenerative progression. Through miRNA sponging, circRNAs participate in the fine-tuning of fundamental processes such as cell proliferation, apoptosis, and differentiation (32-34). Therefore, the miRNA sponging activity of circRNAs enables these molecules as crucial nodes in post-transcriptional regulatory networks, and their dysfunctions directly contribute to disease states.

Regulation of Transcription and Gene Expression

In addition to circRNAs' cytoplasmic roles, numerous circRNAs function within the nucleus as direct regulators of transcription. This activity is primarily attributed to specific subclasses such as ciRNAs and EIciRNAs. One of the mechanisms for regulating transcription involves the direct/physical interaction with the transcription machinery. For

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example, EIciRNAs can bind to U1 snRNP and RNA Polymerase II to stimulate the transcription of their parent genes. An alternative is an indirect mechanism, which involves epigenetic remodeling. Nuclear circRNAs can serve as scaffolds that recruit epigenetic machinery enzymes like TET1, leading to DNA demethylation at promoter regions and subsequently inducing changes in gene expression, a process frequently dysregulated in cancer (35, 36). Thus, in both direct transcriptional enhancement and epigenetic modifications, nuclear circRNAs represent a crucial link between the non-coding circRNAs and the control of fundamental genetic programs.

Interaction with RNA-Binding Proteins

circRNAs can directly interact with RBPs, influencing processes ranging from RNA splicing to translation, thereby functioning as potent regulators. They can regulate gene expression by modulating splicing factor activity. For example, circRNAs can change splice isoform ratios by binding to proteins like hnRNPM or SRSF1, as implicated in cancer pathophysiology. Another prominent example is circFOXO3, which forms a complex with the senescence-associated proteins p21 and CDK2, effectively inhibiting cell cycle progression. These ribonucleoprotein complexes allow circRNAs to impact diverse cellular pathways, including angiogenesis and metastasis (32, 37). Thus, circRNAs emerge as key mediators of protein function and cellular fate by serving as molecular scaffolds or decoys for RBPs.

Protein Translation

Despite their classification as non-coding RNAs, a paradigm-shifting function of circRNAs is their capacity to be translated into protein. Driven by internal IRES elements or m^6A modifications, a subset of circRNAs can synthesize functional peptides and proteins. A prominent translated example of circRNAs is circZNF609, which is translated into a protein in a cap-independent manner and plays a role in myoblast proliferation. The production of such novel polypeptides contributes to proteome diversity and influences critical cellular signaling pathways (14). This recently recognized function vastly expands the biological relevance of circRNAs, positioning them as direct contributors to the proteome, especially under specific physiological and pathological conditions like stress and cancer.

Function as Protein Decoys and Scaffolds

Beyond interacting with RNA-binding proteins, a critical function of circRNAs is to exert their functions by protein decoying or scaffolding, directly modulating protein activity and localization. In this manner, circRNAs can sequester specific proteins, thus preventing them from interacting with their usual targets or their assemblies into functional protein complexes. Some circRNAs can scaffold enzymes with their associated substrates or control transcription factor activity. By decoying and scaffolding proteins, circRNAs can efficiently regulate signaling pathways, influencing critical processes such as cell proliferation, senescence, and DNA damage response (34, 35).

Epigenetic Regulation

An emerging function for circRNAs is playing a role in the epigenetic regulation, as they can act as guide molecules for DNA-modifying enzymes. Specifically, circRNAs can recruit enzymes such as TET demethylases or DNMT methyltransferases to the promoter regions of the genes, leading to targeted methylation pattern modifications in the DNA, causing gene expression alterations. A key example of epigenetic regulation of circRNAs is circ-0001946, which has been shown to suppress metastasis via modulating DNA methylation patterns of specific tumor suppressor genes (35). The capacity to directly regulate epigenetic modifications by circRNAs underscores a fundamental mechanism by which long-term changes can occur in the cell behavior during disease progression.

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Transcriptional Regulation

Certain circRNAs localized in the nucleus, such as circular intronic RNAs (ciRNAs) and EIciRNAs, regulate transcription of their parental genes by interacting with RNA Polymerase II (Pol II) and U1 small nuclear ribonucleoprotein (snRNP) at promoter regions. For example, ci-ankrd52 and ci-sirt7 accumulate at transcription sites and promote Pol II transcription elongation. CircRNAs like circPAIP2 and circEIF3J enhance transcription by connecting Pol II with U1 snRNP, positively regulating gene expression (34, 38).

Epigenetic Regulation

Nuclear circRNAs can modulate epigenetic states by recruiting chromatin modifiers to gene promoters. CircFECR1 recruits the TET1 demethylase to demethylate CpG regions in the DNMT1 promoter, leading to altered DNA methylation and changes in gene expression relevant to cancer invasiveness. This exemplifies how circRNAs influence transcription through epigenetic mechanisms (34).

Regulation of mRNA Stability and Splicing

Nuclear circRNAs can regulate the balance between circular and linear mRNA isoforms through competition in splicing, and some mediate mRNA degradation by recruiting exon junction complexes (EJCs) to target mRNAs, activating a nonsense-mediated decay (NMD)-like pathway. For example, circHOMER1 regulates its cognate mRNA HOMER1B via sequence complementarity and binding of RNA-binding proteins near the 3' UTR, affecting mRNA stability (39).

Modulation of Nuclear Export

The nuclear export of circRNAs is controlled by RNA helicases such as DDX39A and DDX39B, which recognize circRNAs of different lengths, escorting them through the nuclear pore complex. This export process influences the cellular distribution and subsequent cytoplasmic functions of circRNAs (38).

Table 4. CircRNA functions and mechanisms.

Nuclear Function	Description	Examples / Notes
miRNA Sponge	Binds and sequesters miRNAs to release target mRNAs	ciRS-7 sponges miR-7
Transcription Regulation	Enhances or suppresses transcription via promoter binding	circFECR1 recruits TET1 for promoter demethylation
RNA-Binding Protein Interaction	Modulates splicing, stability, translation of RNAs	circURI1 interacts with hnRNPM affecting VEGFA splicing
Protein Translation	Codes for peptides/proteins from circRNA	Emerging evidence in cancer and brain
Epigenetic Regulation	Recruits DNA demethylases to modify gene expression	circFECR1 in breast cancer
Transcriptional Regulation	Interact with Pol II and U1 snRNP to promote transcription	ci-ankrd52, circPAIP2, circEIF3J
Epigenetic Regulation	Recruit chromatin modifiers like TET1 for DNA demethylation	circFECR1 in cancer
mRNA Stability and Splicing	Compete with linear splicing and induce mRNA degradation	circHOMER1 regulates HOMER1B mRNA
Nuclear Export	Mediated by DDX39A/39B helicases for circRNA transport	Length-dependent export of short and long circRNAs

Translation of circRNAs

CircRNAs can be translated into proteins through cap-independent mechanisms, a recently well-established function contrary to their prior classification as non-coding RNAs. Recent studies provide insights into the molecular mechanisms that enable circRNA translation. Here, the circRNA translation features are discussed.

Cap-Independent Translation Initiation

Unlike linear mRNAs, circRNAs lack 5′ caps and 3′ poly-A tails, precluding canonical cap-dependent

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ribosome recruitment. Instead, circRNAs initiate translation via internal IRESs, which are internal RNA sequences that recruit ribosomes directly to the internal positions on the RNA to be translated. The IRES-driven translation involves eukaryotic initiation factors such as eIF4G2, eIF3, and RNA helicases to induce translation complex assembly (6, 40).

Role of m^{6}A Modification

m^{6}A modifications near the start codon contribute to translation initiation by recruiting reader proteins (e.g., YTHDF3) that facilitate ribosome assembly on circRNAs, enhancing translation efficiency in a cap-independent manner (40).

Rolling Circle Translation

Some circRNAs lack in-frame stop codons, allowing ribosomes to continuously translate around the circular RNA, producing repeated peptide sequences in a process called rolling circle translation. This mechanism can generate high molecular weight multimeric proteins that may contribute to novel functional roles (41).

Multiple ORFs and Non-AUG Start Codons

CircRNAs can contain multiple open reading frames (ORFs) and initiate translation from non-AUG codons, increasing protein diversity derived from a single circRNA. For example, circZNF609 has been shown to translate multiple proteins using distinct ORFs (6).

Translation Validations

Proteomic data, polysome association, and detection of backsplice junction-specific peptides via mass spectrometry support endogenous translation of many circRNAs in diverse tissues, including cancer and cardiovascular systems. Experimental validations commonly employ reporter assays, western blotting, and mass spectrometry (42, 43)..

Table 5. CircRNA translation features.

Feature	Description	Examples / Notes
Cap-independent initiation	Uses IRES elements to recruit ribosomes internally	eIF4G2, eIF3, ITAFs involved
m^{6}A modifications	m^{6}A sites near start codon promote translation	Reader proteins like YTHDF3
Rolling circle translation	Continuous translation without a stop codon	Produces multimeric repeating peptides
Multiple ORFs / non-AUG starts	Different proteins from single circRNA	circZNF609 produces several proteins
Experimental evidence	Polysome association, mass spectrometry, reporter assays	Verified in glioblastoma, coronary artery disease

Mitochondrial circRNAs (mecciRNAs):

Mitochondrial circRNAs, known as mecciRNAs, are a distinct class of circular RNAs generated from the mitochondrial genome that play a crucial role in promoting mitochondrial function to support cell migration and metastasis (20). Unlike many nuclear circRNAs, certain mecciRNAs have been demonstrated to encode functional peptides within the mitochondria, adding a novel dimension to the regulatory landscape of mitochondrial biology (44). Their expression is induced under cellular stress conditions, such as during the epithelial-

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mesenchymal transition, where they facilitate the mitochondrial import of nuclear- encoded proteins by acting as molecular chaperones. This process enhances mitochondrial oxidative phosphorylation, thereby providing the necessary energy for cancer cell invasion and metastasis. For example, the specific mecciRNA mecciND5 was shown to be upregulated in metastatic breast cancer models, where it is essential for fueling cell motility and metastatic progression (20, 45, 46).

Biomedical Relevance

CircRNAs exhibit cell-, tissue-, and disease-specific expression profiles, making them attractive biomarkers. Numerous studies have linked circRNAs to malignancies, neurological and cardiovascular diseases (10). Moreover, the stability and modifiability of circRNAs make them promising platforms for therapeutic applications, such as vaccine vectors (47).

CircRNAs Medical Applications

CircRNAs have emerged as promising molecular biomarkers in laboratory medicine, particularly for diagnosis, prognosis, and monitoring of disease progression. They are characterized by their stability, conservation, and tissue specificity, making them superior to linear RNAs for clinical applications. CircRNAs have gained significant attention, particularly for their roles as biomarkers and therapeutic targets in various diseases, including cancer, neurodegenerative disorders, and bone-related diseases. Moreover, engineered circular RNAs are being explored for vaccine design and RNA-based therapies. Some examples of key recent uses of circRNAs in medicine include the following:

1. "CircRNAs: functions and emerging roles in cancer and immunotherapy" discusses the functional roles of circRNAs in cancer progression and their potential in cancer immunotherapy (38).

2. "Research progress of circRNAs in bone-related diseases" covers the involvement of circRNAs in bone diseases and their therapeutic implications (48).

3. "Regulatory mechanism of circular RNAs in brain and neurodegenerative diseases" explores the role of circRNAs in neurodegenerative disorders, highlighting their regulatory mechanisms in brain function (32).

4. A review on cellular functions and biomedical applications of circular RNAs elaborates on their broad potential in medicine and new biomedical applications (49).

5. Insights into circRNAs in cancer stem cells demonstrate their significance in cancer biology and treatment prospects (50).

CircRNAs in Diagnosis

CircRNAs have shown potential as diagnostic biomarkers in various diseases, especially cancers. For example, in hematological tumors, circRNAs have demonstrated a high diagnostic accuracy with a pooled area under the curve (AUC) of 0.86 and both sensitivity and specificity around 79%, indicating their ability to distinguish patients with these tumors from healthy individuals effectively. In colorectal cancer, numerous circRNAs have been identified that correlate with disease presence, making them useful for early diagnosis (51).

CircRNAs in Prognosis

CircRNAs can be used for prognostic value. For instance, in hematological malignancies, certain circRNAs correlate with patient outcomes, such as lymphoid leukemia in which circRNAs negatively impacting prognosis (hazard ratio 1.25). In colorectal cancer, specific circRNAs serve as oncogenes or tumor suppressors, influencing disease progression and patient survival (52) .

CircRNAs in Monitoring

CircRNAs circulating in body fluids can be used to monitor disease progression and treatment response. Exosomal circRNAs, which are stable in body fluids

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like blood, provide unique advantages as non-invasive biomarkers, useful for ongoing monitoring of cancers and potentially other diseases (53).

Conclusion

Circular RNAs (circRNAs) are remarkably stable and act as microRNA sponges, interacting with RNA binding proteins, forming RNA-DNA hybrids, undergoing N6A modification, and even being translated into peptides. Their dysregulation has been implicated in various diseases—cancer, neurodegeneration, and cardiovascular disorders—and they show promise as diagnostic biomarkers and therapeutic targets.

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No AI writing assistance was utilized in the production of this manuscript.

Conflict of interest

The authors have no competing interests to disclose.

Ethical considerations

This study was a review based on publicly available published data and did not require ethical approval.

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There are no associated data with this article

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